AmplifyRP® XRT for CGMMV
Validation Report
Cucumber green mottle mosaic virus
Product No. XCS 45702

Test Characteristics

Test Name	Cucumber green mottle mosaic virus	**Test Label**	FAM-labeled target probe
Catalog Number	45702	**Internal Control**	Endogenous
Acronym	CGMMV	**Format**	XRT
Genus	Tobamovirus	**Diluents**	GEB2/PD1
Binomial Name	Tobamovirus viridimaculae	**Sample Dilution**	1:20 (leaf) / 1:10 (seed)

Summary

AmplifyRP XRT for CGMMV is a rapid RNA amplification and detection platform designed for testing cucurbits for Cucumber green mottle mosaic virus. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify CGMMV RNA and an endogenous RNA control at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	91
Correct Diagnoses	91
Percent	100%

Analytical Sensitivity

Analytical Sensitivity:	The assay is 87.5% sensitive between 1 fg/µL and 10 fg/µL. (n=40)
Limit of Detection:	The assay has a 100% detection rate at 10 fg/µL with RNA transcripts. (n=20)
	The assay has a 75.0% detection rate at 1 fg/µL with RNA transcripts. (n=20)

Analytical Specificity

Inclusivity:

Isolates and Geographic Regions Detected:

CGMMV-11 (Taiwan)[1]	CGMMV-1aq (China)[1]
CGMMV-3nj (China)[1]	CGMMV-81-k141 (China)[1]
CGMMV-ABCA13-01 (Canada)[1]	CGMMV-Ah (Israel)[1]
CGMMV-Alm08 (Spain)[1]	CGMMV-Anhui (China)[1]
CGMMV-ATCC®-PV-391™ (Japan)	CGMMV-ATCC®-PV-41™-FD
CGMMV-BG (Taiwan)[1]	CGMMV-BG-SB (Taiwan)[1]
CGMMV-BHZC36029 (China)[1]	CGMMV-C (Taiwan)[1]
CGMMV-C284R (China)[1]	CGMMV-Ca (Canada)[1]
CGMMV-CCMV_TANG (China)[1]	CGMMV-CG001 (CA, USA)[1]
CGMMV-CG002 (CA, USA)[1]	CGMMV-CG003 (CA, USA)[1]
CGMMV-CG004 (CA, USA)[1]	CGMMV-CG005 (Japan)[1]
CGMMV-CG008 (Netherlands)[1]	CGMMV-CG009 (Netherlands)[1]
CGMMV-CG012 (Netherlands)[1]	CGMMV-CG013 (Netherlands)[1]
CGMMV-CG017 (Thailand)[1]	CGMMV-CG018 (Thailand)[1]
CGMMV-CG021 (Netherlands)[1]	CGMMV-CG022 (Netherlands)[1]
CGMMV-CG026 (France)[1]	CGMMV-CG027 (France)[1]
CGMMV-CG031 (Netherlands)[1]	CGMMV-CG032 (Russia)[1]
CGMMV-CG035 (Kuwait)[1]	CGMMV-CG036 (Canada)[1]

Isolates and Geographic Regions Detected:

CGMMV-CG040 (CA, USA)[1]	CGMMV-CG045 (CA, USA)[1]
CGMMV-CG048 (CA, USA)[1]	CGMMV-CG049 (CA, USA)[1]
CGMMV-CG052 (CA, USA)[1]	CGMMV-CG053 (CA, USA)[1]
CGMMV-CG056 (CA, USA)[1]	CGMMV-CG057 (CA, USA)[1]
CGMMV-CJLX28437 (China)[1]	CGMMV-CPVC-0121
CGMMV-eWT (China)[1]	CGMMV-GDLZ (China)[1]
CGMMV-JD8 (China)[1]	CGMMV-JN (China)[1]
CGMMV-LN (China)[1]	CGMMV-MC-1 (Russia)[1]
CGMMV-NT (Australia)[1]	CGMMV-NT-2014-02 (Australia)[1]
CGMMV-NT-2017-02 (Australia)[1]	CGMMV-NT-2017-03 (Australia)[1]
CGMMV-NT-2019-07 (Australia)[1]	CGMMV-NT-2019-08 (Australia)[1]
CGMMV-NT-2020-05 (Australia)[1]	CGMMV-OM-1 (South Korea)[1]
CGMMV-ON5 (Canada)[1]	CGMMV-ON6 (Canada)[1]
CGMMV-PV-0375 (Germany)[1]	CGMMV-PV-1333 (Israel)
CGMMV-QLD-2019-02 (Australia)[1]	CGMMV-Rd (Israel)[1]
CGMMV-SD cucumber (China)[1]	CGMMV-SD Luffa (China)[1]
CGMMV-SI-2015-01[1]	CGMMV-SI-2016-01[1]
CGMMV-SluRVP22_08 (Russia)[1]	CGMMV-spider132626 (China) [1]
CGMMV-TY (Israel)[1]	CGMMV-TZ4 (China)[1]
CGMMV-WA-2 (Australia)[1]	CGMMV-WA-3 (Australia)[1]
CGMMV-WA-6 (Australia)[1]	CGMMV-WA-7 (Australia)[1]
CGMMV-WM-1 (South Korea)[1]	CGMMV-WM-2 (South Korea)[1]
CGMMV-XG (China)[1]	CGMMV-XS7 (China)[1]
[1]Predicted detection by *in silico* analysis only	

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
None Known	

Does Not Cross-react With:

Virus Name	Species Name
African eggplant-associated virus (AEaV)[1,2]	N/A
Bell pepper mottle virus (BPeMV)[1]	Tobamovirus maculacapsici
Brugmansia mild mottle virus (BrMMV)[1]	Tobamovirus brugmansiae
Cactus mild mottle virus (CMMoV)[1]	Tobamovirus cacti
Clitoria yellow mottle virus (CliYMMV)[1]	Tobamovirus clitoriae
Cucumber Bulgarian virus (CBLV)[1]	Tombusvirus bulgariaense
Cucumber fruit mottle mosaic virus (CFMMV)[1]	Tobamovirus maculafructi
Cucumber leaf spot virus (CLSV)	Aureusvirus cucumis
Cucumber mosaic virus (CMV)	Cucumovirus CMV
Cucumber mottle virus (CMoV)[1]	Tobamovirus cucumeris
Cucumber necrosis virus (CNV)	Tombusvirus cucumis
Cucumber soil-borne virus (CuSBV)	N/A
Cucumber vein yellowing virus (CVYV)	Ipomovirus cucumisvenaflavi
Cucumber vein-clearing virus (CuVCV)[1]	Carlavirus cucumis

Does Not Cross-react With:

Virus Name	Species Name
Cucurbit aphid-borne yellows virus (CABYV)	Polerovirus CABYV
Cucurbit leaf crumple virus (CuLCrV)[1]	Begomovirus cucurbitae
Cucurbit mild mosaic virus (CuMMV)[1]	Fabavirus cucurbitaceae
Cucurbit yellow stunting disorder virus (CYSDV)[1]	Crinivirus cucurbitae
Frangipani mosaic virus (FrMV)	Tobamovirus frangipani
Hibiscus latent Fort Pierce virus (HLFPV)[1]	Tobamovirus fortpiercense
Hibiscus latent Singapore virus (HLSV)[1]	Tobamovirus singaporense
Kyuri green mottle mosaic virus (KGMMV)	Tobamovirus kyuri
Maracuja mosaic virus (MarMV)[1]	Tobamovirus maracujae
Melon aphid-borne yellows virus (MABYV)[1]	Polerovirus MABYV
Melon mild mottle virus (MMMoV)[1]	Nepovirus cucumis
Melon necrotic spot virus (MNSV)	Gammacarmovirus melonis
Melon rugose mosaic virus (MRMV)[1]	Tymovirus melonis
Melon yellowing-associated virus (MYaV)[1]	Carlavirus melonis
Obuda pepper virus (ObPV)	Tobamovirus obudae
Odontoglossum ringspot virus (ORSV)	Tobamovirus odontoglossi
Paprika mild mottle virus (PaMMV)	Tobamovirus paprikae
Passion fruit mosaic virus (PafMV)[1]	Tobamovirus passiflorae
Pepper mild mottle virus (PMMoV)	Tobamovirus capsici
Plumeria mosaic virus (PluMV)[1]	Tobamovirus plumeriae
Rattail cactus necrosis-associated virus (RCNAV)[1]	Tobamovirus muricaudae
Rehmannia mosaic virus (ReMV)	Tobamovirus rehmanniae
Ribgrass mosaic virus (RMV)	Tobamovirus plantagonis
Opuntia chlorotic ringspot virus (OCRSV)[1]	Tobamovirus opuntiae
Squash leaf curl China virus (SLCCNV)[1]	Begomovirus cucurbitachinaense
Squash leaf curl Philippines virus (SLCPHV)[1]	Begomovirus cucurbitaphilippinense
Squash leaf curl virus (SLCV)	Begomovirus cucurbitapeponis
Squash leaf curl Yunnan virus (SLCYNV)[1]	Begomovirus cucurbitayunnanense
Squash mild leaf curl virus (SMLCV)[1]	Begomovirus cucurbitatenuis
Squash mosaic virus (SqMV)	Comovirus cucurbitae
Squash vein yellowing virus (SqVYV)[1]	Ipomovirus cucurbitavenaflavi
Streptocarpus flower break virus (SFBV)	Tobamovirus streptocarpi
Sunn-hemp mosaic virus (SHMV)	Tobamovirus crotalariae
Tobacco latent virus (TLV1)[1]	Tobamovirus latentabaci
Tobacco mild green mosaic virus (TMGMV)	Tobamovirus mititessellati
Tobacco mosaic virus (TMV)	Tobamovirus tabaci
Tomato brown rugose fruit virus (ToBRFV)	Tobamovirus fructirugosum
Tomato leaf curl New Delhi virus (ToLCNDV)[1]	Begomovirus solanumdelhiense
Tomato mosaic virus (ToMV)	Tobamovirus tomatotessellati
Tomato mottle mosaic virus (ToMMV)	Tobamovirus maculatessellati
Tropical soda apple mosaic virus (TSAMV)[1]	Tobamovirus tropici
Turnip vein-clearing virus (TVCV)	Tobamovirus rapae
Ullucus mild mottle virus (UMMV)[1]	Tobamovirus ulluci
Wasabi mottle virus (WMoV)[1]	Tobamovirus wasabi

Does Not Cross-react With:

Virus Name	Species Name
Watermelon bud necrosis virus (WBNV)[1]	Orthotospovirus citrullonecrosis
Watermelon mosaic virus (WMV)	Potyvirus citrulli
Watermelon silver mottle virus (WSMoV)	Orthotospovirus citrullomaculosi
Wild cucumber mosaic virus (WCMV)[1]	Tymovirus cucumis
Yellow tailflower mild mottle virus (YTMMV)[1]	Tobamovirus anthocercis
Youcai mosaic virus (YoMV)	Tobamovirus youcai
Zucchini green mottle mosaic virus (ZGMMV)	Tobamovirus cucurbitae
Zucchini lethal chlorosis virus (ZLCV)[1]	Orthotospovirus cucurbichlorosis
Zucchini shoestring virus (ZSSV)[1]	Potyvirus peporesticulae
Zucchini tigre mosaic virus (ZTMV)[1]	Potyvirus pepotigris
Zucchini yellow fleck virus (ZYFV)[1]	Potyvirus pepo
Zucchini yellow mosaic virus (ZYMV)	Potyvirus cucurbitaflavitesselati
[1]Predicted non-detection by *in silico* analysis only	

Diagnostic Specificity

True Negatives 62
Correct Diagnoses 62
Percent 100%

Selectivity:

No Matrix Effect Observed With:			
Bitter gourd leaves	Bitter gourd seed	Bottle gourd leaves	Bottle gourd seed
Cucumber leaves	Cucumber seed	Melon leaves	Melon seed
Pepino leaves	Pumpkin leaves	Pumpkin seed	Squash leaves
Squash seed	Watermelon leaves	Watermelon seed	Zucchini leaves
The hosts on the above list have been chosen to represent those which historically cause a range of matrix effects, in addition to those expected to be screened for this pathogen. Not all plant species susceptible to this pathogen have been screened, but may still be used with this assay unless otherwise noted below. As with all diagnostic tools, Agdia recommends confirming all results with a secondary detection method before making any economic decisions (ex: discarding plants due to positive test results, etc.).			

Matrix Effect Observed With:			
None Known			

Repeatability

Number of Samples 148
Replicates per Sample 2 - 8
Total Replicates 342
Replicates in Agreement 338
Percent Agreement 98.8%

Reproducibility

Number of Samples 32
Replicates per Sample 3
Number of Operators 4
Total Replicates 384
Replicates in Agreement 364
Percent Agreement 94.8%

Robustness

Planned deviation analysis:
No deviations from the user guide protocol were validated.

Stability:

	1-year stability (accelerated)	Real-time Stability Verification
Positive Sample (High)	Pass	Monitoring
Positive Sample (High)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Negative Sample	Pass	Monitoring
Negative Sample	Pass	Monitoring

Glossary

Diagnostic sensitivity[1]: The percentage of positive samples correctly identified in an experiment with known positive controls.

Diagnostic specificity[1]: The percentage of negative samples correctly identified in an experiment with known negative controls.

Analytical sensitivity[3]: The smallest amount of target that can be detected reliably (this is sometimes referred to as the 'limit of detection')

Analytical specificity[3]: (comprises inclusivity and exclusivity)

Inclusivity[3]: The performance of a test with a range of target isolates covering genetic diversity, different geographical origin and/or hosts associated with the target organism.

Exclusivity[3]: The performance of a test with a range of non-targets (e.g. cross-reaction with closely related organisms, contaminants)

Selectivity[2]: The level of effect that matrices and relevant plant parts have on the performance of the assay.

Repeatability[2]: The agreement between test replicates of the same sample tested by the same operator.

Reproducibility[3]: The ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g. time, users, equipment, location)

Robustness[1,3]: The extent to which varying test conditions (e.g. temperature, volume, change of buffers) affect the established test performance values. May also be referred to as planned deviation analysis.

Stability[1]: The performance of test reagents or controls over time.

References:
[1]Groth-Helms, D., Rivera, Y., Martin, F. N., Arif, M., Sharma, P., Castlebury, L. A. (in press). Terminology and Guidelines for Diagnostic Assay Development and Validation: Best Practices for Molecular Tests. PhytoFrontiers.
[2]Eads, A., Groth-Helms, D., Davenport, B., Cha, X., Li, R., Walsh, C., Schuetz, K., (in press). The Commercial Validation of Three Tomato Brown Rugose Fruit Virus Assays. PhytoFrontiers.
[3]EPPO (2018) PM 7/76 (5) Use of EPPO Diagnostic Standards, EPPO Bulletin 48, 373– 377.

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